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A Molecular Assessment of Phylogenetic Relationships and Lineage Diversification Within the Family Salamandridae (Amphibia, Caudata)

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Abstract

Phylogenetic relationships among species of the salamander family Salamandridae are investigated using nearly 3000 nucleotide bases of newly reported mitochondrial DNA sequence data from the mtDNA genic region spanning the genes $tRNA^{Leu}$-COI. This study uses nearly comprehensive species-level sampling to provide the first complete phylogeny for the Salamandridae. Deep phylogenetic relationships among the three most divergent lineages in the family – *Salamandrina terdigitata*, a clade comprising the "True" salamanders, and a clade comprising all newts except *S. terdigitata* – are difficult to resolve. However, most relationships within the latter two lineages are resolved with robust levels of branch support. The genera *Euproctus* and *Triturus* are statistically shown to be nonmonophyletic, instead each contains a diverse set of lineages positioned within the large newt clade. The genus *Paramesotriton* is also resolve as a nonmonophyletic group, with the newly described species *P. laoensis* constituting a divergent lineage placed in a sister position to clade containing all *Pachytriton* species and all remaining *Paramesotriton* species. Sequence divergences between *P. laoensis* and other *Paramesotriton* species are as great as those comparing *P. laoensis* and species of the genera *Cynops* and *Pachytriton*. Analyses of lineage diversification across the Salamandridae indicate that, despite its exceptional diversity, lineage accumulation appears to have been constant across time, indicating that it does not represent a true species radiation.

1. Introduction

The salamander family Salamandridae, with its 15 genera and 63 recognized species, represents one of the most diverse groups of extant salamanders. Salamandrid diversity covers the largest geographic distribution of any salamander family and spreads across the holarctic continents of Asia, Europe, and North America with a small and recent spread into North Africa. The Salamandridae comprises two main groups: (1) the traditionally recognized newts (salamanders with rough keratinized skin) and (2) the "true" salamanders (smooth-skinned salamandrids). The Salamandridae has been proposed to contain sets of evolutionary radiations (Wake and Ozeti, 1969) that have diversified as a function of evolution in both terrestrial and aquatic environments, potentially through the evolution of a variety of feeding morphologies (Ozeti and Wake, 1969), and courtship behaviors (Salthe, 1967). The Salamandridae as a radiation or set of radiations implies that there has been an increase in the rate of accumulation of lineages within these radiations (Schluter, 2000). However, there has been little exploration of the tempo of lineage diversification across the entire salamandrid family (but see the lower level studies of Weisrock et al., 2001; Steinfartz et al., 2000). The fossil record is sparse for this family meaning that insights into the rates of lineage formation will need to come from alternative sources.

Phylogenies have become an important source of information for studying the tempo of lineage diversification (Slowinski and Guyer, 1989; Mooers and Heard, 1997; Nee et al., 1994; Sanderson and Donoghue, 1996). By plotting lineage accumulation as a function of time a visual perspective can be gained into the rates of diversification. The integration of this information with null models of the of the birth and death of lineages (Nee et al., 1992) permit hypotheses of lineage diversification over time to be statistically tested (Paradis, 1997; Pybus and Harvey,

2000; Pybus et al., 2002). These phylogenetic approaches have yielded important insight in the tempo of evolutionary diversification in a number of organismal groups including iguanian lizards (Harmon et al., 2003), Marine fish (Ruber and Zardoya, 2005), and bryophyte mosses (Shaw et al., 2003).

No single study has comprehensively investigated phylogenetic relationships among all salamandrid species. The most complete phylogenetic study of the family was conducted by Titus and Larson (1995) using a combination of morphological and mitochondrial DNA (mtDNA) (12S and 16S rDNA and the intervening $tRNA^{Val}$) characters from 18 species. This study provided strong support for the monophyly of the Salamandridae and for some intergeneric groupings. Furthermore, the monophyly of the genera *Mertensiella* and *Triturus* was statistically rejected. However, there was little support for many basal relationships within the family, particularly for the placement of the monotypic newt genus *Salamandrina*. Titus and Larson (1995) characterized *Salamandrina* as a divergent lineage in the family, but the deep phylogenetic branching pattern among *Salamandrina*, the true salamanders, and the remaining newts was effectively left unresolved.

Phylogenetic relationships within many salamandrid groups have received considerable attention (e.g. Caccone et al., 1997; Chan et al., 2001; Lu et al., 2004; Steinfartz et al., 2000, 2002; Veith et al., 2004; Weisrock et al., 2001), yet many species relationships still require further resolution. Evolution of the genus *Triturus* has been studies extensively (Halliday and Arano, 1991), yet phylogenetic resolution among species has been difficult to achieve, even from a host of morphological, molecular, and behavioral data (Giacomo and Balletto, 1988; Macgregor et al., 1990; Zajc and Arntzen, 1999). Monophyly of the genus *Triturus* was rejected by the mtDNA studies of Titus and Larson (1995), based on two species. However, studies

using more comprehensive ingroup sampling, but limited outgroup sampling have found *Triturus* to be either monophyletic or paraphyletic (e.g. Zajc and Arntzen, 1999). Recent studies of the genus *Euproctus* indicate that it also may not be nonmonophyletic (Caccone et al., 1994, 1997), and instead may represent a set of distantly related lineages closely intertwined with species of *Triturus*. A thorough phylogenetic assessment of the these genera, as well as most other salamandrid lineages may be better resolved through comprehensive sampling of the entire family.

In this study we use nearly comprehensive taxon sampling in conjunction with new and previously published mtDNA sequence data to address both the deep phylogenetic relationships among major lineages of salamandrids and the relationships among the more recently derived lineages within deeply diverged groups. The resulting phylogenies are then used to address the tempo of lineage diversification across the history of the Salamandridae.

2. Materials and Methods

2.1 Taxon Sampling and Data Collection

This study used approximately 2700 bases of new mtDNA sequence data collected from 96 individuals including 60 of the 64 recognized salamandrid species and outgroups. Four salamandrid species were not included: *Triturus helveticus*, *Triturus italicus*, *Cynops chenggongensis*, and *Cynops wolterstorfii*. The latter species is considered to be recently extinct (Zhao, 1998). We follow the taxonomic suggestion of Veith and Steinfartz (2004) in placing *Mertensiella luschani* in a new genus, *Lyciasalamandra*, based on mtDNA-based statistical support for the nonmonophyly of the previously recognized genus *Mertensiella* (Weisrock et al. 2001) and corroborating allozyme-based genetic evidence (Veith and Steinfartz, 2004).

Sequence data was collected from a contiguous block of genes including $tRNA^{Leu}$, ND1, $tRNA^{Ile}$, $tRNA^{Gln}$, $tRNA^{Met}$, ND2, $tRNA^{Trp}$, $tRNA^{Ala}$, $tRNA^{Asn}$, the origin for light strand replication (O_L), $tRNA^{Cys}$, $tRNA^{Tyr}$, and COI (hereafter referred to as the $tRNA^{Leu}$-COI genic region). All genes included are full length except for COI, which contained approximately 30 bases of 5' partial sequence. This gene region is similar to the one used in an earlier study of the "true" salamanders (Weisrock et al., 2001), except that it contains approximately 670 additional bases of sequence completing the 5' portion of the ND1 gene and the preceding $tRNA^{Leu}$ gene. This additional sequence data was generated for individuals used in Weisrock et al., 2001 and added to their already available GenBank sequence. DNA extraction, PCR, and sequencing methods were performed as in Weisrock et al. (2001) with the exception that most sequencing reactions were performed using a Big-Dye Terminator Ready-Reaction Kit (Perkin-Elmer) and run on either an ABI™ (PE Applied Biosystems, Inc.) 373A automated DNA sequencer or an MJ Research BaseStation.

We also included GenBank and published mtDNA sequence data from two additional gene regions for use in combined phylogenetic analyses with our data. This included a data set of 12S-$tRNA^{Val}$-16S sequence for 32 ingroup taxa and 5 outgroups (Caccone et al., 1994; Steinfartz et al., 2002; Titus and Larson, 1996; Zajc and Arntzen, 1999) and a data set of cytochrome *b* sequences for 32 ingroup taxa and 2 outgroups [Alexandrino et al., 2002; Caccone et al., 1994; Chan et al., 2001; Chippindale et al., 2001; García-París et al., 2002; Hedges et al., 1992; Tan and Wake, 1995). Sequences in the 12S-$tRNA^{Val}$-16S range from approximately 300-1000 bp in length. Sequences in the cytochrome *b* data set range from approximately 380-700 bp in length. See Appendix 1 for more detail regarding these sequences. Additional

mitochondrial regions are available in GenBank, but provide limited sampling across the family and were not used in this study.

2.2 Phylogenetic Analysis

Alignment of the mtDNA sequence was performed manually using amino-acid sequence translations for protein-coding genes and secondary-structural models for tRNA genes (Kumazawa and Nishida, 1993). Length-variable regions whose alignment was ambiguous, including many loop regions of tRNAs and much of the origin for light-strand replication (O_L), were excluded from phylogenetic analyses.

Phylogenetic trees were generated under both parsimony and Bayesian criteria in the analysis of our new data set as well as in combined analyses with previously published sequence data. Parsimony analysis was performed using PAUP* v4.0 (Swofford, 2002). A heuristic search option with 100 random-addition replicates was used with equal weighting of all characters and TBR branch swapping. To assess support for branches in parsimony trees, bootstrap percentages (BP) were calculated using 1000 bootstrap replicates with 100 random additions per replicate. Bayesian phylogenetic analysis was performed using the parallel-processor version of MrBayes v3.04 (Altekar et al. 2004). Bayesian analysis of the new mtDNA sequence data was performed by treating all sequence data as a single data partition and by using a three data partition format: ND1, ND2+COI, and tRNA sequence data. Combined analysis of the new data and previously published sequence used five data partitions: ND1, ND2+COI, Cytb, 12S+16S, and tRNA sequence data. All analyses used four Markov chains with the temperature profile at the default setting of 0.2. The best-fit evolutionary model used was determined by likelihood-ratio tests as implemented in MODELTEST version 3.06 (Posada and Crandall,

1998). Uniform, default priors were used for model parameter estimates, and random trees were used to start each Markov chain. A molecular clock was not enforced. Two million generations were run with a tree sampling taken every 1000th generation for a total of 2,000 trees. The program TRACER (Rambaut and Drummond, 2003) was used to determine when the –Log Likelihood (-lnL) of sampled trees reached a stationary distribution. The first one million generations were discarded as "burn in". Sampled trees from the posterior distribution were parsed with MrBayes to construct a phylogram based upon mean branch lengths and to calculate the posterior probabilities (PP) of all branches using a majority-rule consensus approach. To account for the possibility that individual analyses may not be converging upon the optimal posterior distribution, two additional independent runs were performed for each data set using identical conditions. Likelihood values, tree topology, branch lengths, and posterior probabilities were compared across the replicated runs to verify that similar results were being achieved.

Alternative phylogenetic topologies were tested using the Templeton Test (Templeton, 1983) and the Shimodaira and Hasegawa (SH) test using 1000 RELL bootstrap replicates (Goldman et al., 2000; Shimodaira and Hasegawa, 1999), both implemented in PAUP* v4.0. To perform the SH tests, a maximum-likelihood tree was found in an unconstrained analysis treating the entire data set as a single partition and using the best-fit model of evolution. Model parameter estimates were set using mean parameter estimates from an unpartitioned Bayesian phylogenetic analysis. The unconstrained ML tree was compared to a ML tree favoring a particular topological constraint. To expedite the likelihood search process for constrained ML trees, we preserved branches in the constraint tree that had Bayesian posterior probabilities ≥0.95 and were not directly involved with the alternate branching event.

2.3 Diversification Analyses

To obtain ultrametric trees for use in diversification analyses, trees from the Bayesian posterior distribution were subjected to lineage rate smoothing using a penalized likelihood procedure (Sanderson, 2002). All outgroup taxa were pruned from the trees as well as nine ingroup sequences that were shallowly diverged (<1% pairwise sequence divergence) from other members of their clades. Optimal smoothing values were obtained using a cross-validation procedure using the truncated Newton method.

To obtain a visual perspective of the rate of accumulation of lineages over time we constructed lineage-through-time (LTT) plots (Nee et al., 1992) for ten trees sampled from the posterior distribution (trees 1, 101, 201, 302, 401, 501, 601, 700, 801, and 900) using the program LTT (written by L. Harmon). For each of these ten trees we quantified the LTT patterns through the use of the γ statistic (Pybus and Harvey, 2000; Pybus et al., 2002). Trees exhibiting increased speciation rates during all or a portion of their history (or decreased extinction rates) are expected to produce concave LTT plots and a $\gamma > 0$, while trees that exhibit a decrease in speciation rates (or an increased extinction rates) are expected to produce a convex LTT plot and a $\gamma < 0$. In addition to assessing diversification across the entire tree we also investigated patterns of lineage accumulation in the early evolutionary history of the Salamandridae by calculating γ for the first 2/3 of each tree (starting from the deepest node to a cumulative branch length of 0.67). Gamma statistics were used in a constant-rate (CR) test (Pybus and Harvey, 2000) to assess whether the rates of lineage accumulation over time have changed. Because we have nearly complete taxon sampling for the family the CR test is appropriate without having to perform a Monte Carlo simulation to account for missing lineages. Under the CR test a constant-rates model of lineage diversification can be rejected when $\gamma <$-

1.645 (Pybus et al., 2002). The CR test assumes that lineage diversification occurs equally across the phylogeny; therefore, we used the relative-cladogenesis statistic (Pk) as implemented in the program End-Epi v1.0.1 (Rambaut et al., 1997) to identify ancestral branches that have significantly higher than expected rates of cladogenesis. This test calculates the probability (Pk) that a particular lineage at time *t* will have *k* tips given the total number of tips at time 0 (the present).

3. Results

3.1 New tRNA^{Leu}-COI Salamandrid Phylogeny

The sequence alignment of the tRNA^{Leu} -COI genic region after exclusion of ambiguously aligned characters resulted in a total of 2607 characters for phylogenetic analysis (1705 variable; 1483 parsimony informative). Likelihood-ratio tests choose the General Time-Reversible (GTR) model for the total data set with a proportion of sites being invariable (I) and rate heterogeneity across sites (Γ). The individual ND1 and ND2+COI data partitions are also favored by the GTR+I+Γ model. The tRNA partition was found to be best fit to an HKY+I+Γ model. Bayesian analysis of the unpartitioned tRNA^{Leu} -COI data results in a posterior distribution with an average log likelihood (lnL) of –62785.3. A Bayesian analysis treating the ND1, ND2+CO1, and tRNA data as separate partitions produces a posterior distribution with an average lnL of –62676.71. Mean model parameter estimates of each data partition calculated from the Bayesian posterior distribution are presented in Table 3. The unpartitioned and tri-partitioned Bayesian analyses produce similar topologies and a generalized partitioned Bayesian consensus phylogram is presented (Fig. 1). Parsimony analysis produces 14 trees of 14198 steps in length and a strict consensus tree produces a topology (Fig. 2) that is very similar to the

partitioned Bayesian tree. The resolution and relationships of major clades between the two trees are nearly identical except for the placement of *Salamandrina terdigitata*, which is placed as the sister lineage to the "true" salamanders in the Bayesian consensus tree, but is placed as the sister lineage to a clade containing all remaining newts in the parsimony consensus tree. The partitioned Bayesian analysis finds strong support for the clade containing *Salamandrina* and the "true" salamanders (PP=0.95); however this support decreases in the unpartitioned analysis (PP=0.84). Parsimony analysis poorly supports the monophyly of all newts (BP<50%). Statistical tests of alternative phylogenetic relationships using both the SH test and Templeton test were not significant (Table 2). Results among and within major salamandrid clades were highly congruent between the Bayesian and Parsimony analyses. Bayesian consensus phylograms for these clade are presented in Figures 3 and 4 with posterior probabilities and parsimony bootstrap values mapped to individual branches.

3.2 Combined mtDNA Phylogeny

The inclusion of additional cytochrome *b* and 12S-tRNA^{Val}-16S mtDNA sequence from GenBank resulted in a combined character matrix of 4529 nucleotides of which 4134 were included in analyses (2405 variable; 2024 parsimony informative). The cytochrome *b* and 12S+16S data sets are each favored by a GTR+I+Γ model of evolution. An expanded tRNA data set including tRNA^{Val} is favored by the HKY+I+Γ model. Bayesian analysis of a five partition data set (ND1, ND2+COI, tRNAs, Cyt b, and 12S+16S rDNAs) produces a posterior distribution with an average lnL of –74464.94. Parsimony analysis of the combined data results in a single tree of 16692 steps in length. Inclusion of this extra data does little to change the branching structure of the tRNA^{Leu}-COI-based analyses, nor does it improve branch support for some

important relationships. For example, the combined-data Bayesian tree places *Salamandrina* as the sister lineage to a clade of "true" salamanders with a PP of 0.72, which is lower than the PP for this relationship in the partitioned Bayesian analysis of the ND1-ND2-COI data. Parsimony analysis of the combined data again places *Salamandrina* as the sister lineage to all remaining newts with a bootstrap of 70%.

3.2 Analysis of Lineage Diversification

The relative cladogenesis statistic does not reject the hypothesis of equal diversification through time for any branch in the PL-smoothed Bayesian consensus tree. Lineage-through-time plots for 10 trees sampled from the Bayesian posterior distribution produce similar patterns (Fig. 5). All trees exhibit a slightly convex pattern early in the history of the salamandrid diversification, but the latter portions of the LTT curves do not diverge substantially from a pattern expected under a pure-birth model (diagonal dashed line in Fig. 5). Gamma statistics calculated for the total phylogenetic history of each tree yield an average γ of –0.1397 (range –0.7317 to 0.4539) (Table 4). Gamma statistics calculated for the first 2/3 of the phylogenetic history of each tree yield a more negative average γ of –0.8956 (range –1.2302 to -0.5452) (Table 4), congruent with the LTT curves yielding a more convex pattern earlier in salamandrid history. However, despite the negative γ measured for most tree trees, no measure of γ rejects a constant rate of lineage accumulation over time.

4. Discussion

4.1. Major Salamandrid Lineages and Their Phylogeny

Our results provide the most comprehensive view to date of salamandrid phylogeny. We expand on previous phylogenetic assessments of salamandrid phylogeny by generating a data set that includes nearly all recognized species of the family and a number of intraspecifically divergent samples. Analyses of these data provide robust relationships for many of the deep relationships within the family as well as many of the more terminal relationships within major salamandrid lineages. We provide discussion of these relationships by first focusing on the resolution of phylogenetic relationships among major lineages. We then discuss relationships among taxa within these lineages and close with a discussion regarding lineage diversification in the Salamandridae.

The results presented here are in agreement with previous higher-level studies of salamandrid phylogeny (Titus and Larson, 1995) in characterizing deep divergences among three major lineages: (1) the Italian endemic *Salamandrina terdigitata*, (2) the mostly European "true" salamanders, and (3) and a Holarctic distributed clade of all newts excluding *S. terdigitata*. The latter two clades are each individually strongly supported in both Bayesian and parsimony analyses (Figs. 1 and 2). Monophyly of the "true" salamanders has been supported by previous molecular studies (Veith et al., 1998; Weisrock et al., 2001). Similarly, a newt clade that excluded *Salamandrina* was resolved in the trees of Titus and Larson (1995); however, branch support was low (BP=69-73%). Our results strongly support the resolution of these three major lineages, but with the inclusion of a comprehensive sampling effort across the entire family.

Our results do not find overwhelming and convincing support for one of the most important aspects of salamandrid evolution: the phylogenetic placement of *Salamandrina*. Partitioned Bayesian analysis of the ND1-COI mtDNA sequence provide potentially strong support for the placement of *Salamandrina* as the sister lineage to the "true" salamanders

(PP=0.95), but support decreases in the unpartitioned analysis of this data (PP=0.84) and in the combined and partitioned analysis of all mtDNA sequence data (PP=0.72). Alternatively, parsimony analysis of the ND1-COI and total mtDNA data sets weakly support the placement of *Salamandrina* as the sister lineage to all remaining newts (BP <50% and 70%, respectively). The Bayesian placement of *Salamandrina* is concordant with previous morphology-based phylogenies of the family (Titus and Larson, 1996; Wake and Özeti, 1969). Most of these characters were based on hyobranchial morphology, an important structural complex due to its role in feeding in terrestrial (*Salamandrina* and the "true" salamanders) versus aquatic (all remaining newts) environments (Özeti and Wake, 1969). It is possible that many of these characters are not independent, but instead evolve as part of a linked and complex character structure. Selection for feeding in a terrestrial environment could have acted to produce convergent morphologies in *Salamandrina* and the "true" salamanders. This scenario fits with the evolutionary view from previous parsimony-based analyses of combined mtDNA and morphology data, which resolve a clade of all newts including *Salamandrina*, and indicate that morphological character support is weak (Titus and Larson, 1995).

In our analyses statistical tests cannot reject alternative placements of *Salamandrina* under either phylogenetic criterion indicating that neither the Bayesian nor parsimony analyses overwhelmingly support one phylogenetic scenario over the other. Therefore, we suggest caution in interpreting the Bayesian results as support for a relationship between *Salamandrina* and the "true" salamanders. While Bayesian analysis can outperform parsimony analysis in deep phylogenetic reconstruction (Weisrock et al. In Press), it can also be highly sensitive to model parameterization (Buckley, 2002) and saturated data (Weisrock et al. In Press). Consequently,

without verification through additional independent genomic markers, we consider the phylogenetic placement of *Salamandrina* to remain unresolved.

4.2. Phylogenetics of the "true" Salamanders

Relationships within the clade of "true" salamanders support previous molecular studies of this group with a primary phylogenetic split between a clade containing *Chioglossa* and *Mertensiella* and a clade containing the genera *Lyciasalamandra* and *Salamandra* (Figs. 1, 2; Veith et al., 1998; Weisrock et al., 2001). *Lyciasalamandra* and *Salamandra* are resolved as phylogenetically divergent and well supported clades. Previous phylogenetic studies within *Salamandra* have not provided robust resolution among species (Garcia-Paris et al., 2003; Steinfartz et al., 2000). Steinfartz et al. (2000) used a phylogeographic approach to resolve a number of geographically defined lineages that corresponded to recognized taxonomic groups. However, there was little resolution among these lineages, which was hypothesized to be the result of diversification over a relatively short period of time. Our results find strong support for most relationships among species of *Salamandra*. Bayesian and parsimony analyses yield congruent topologies with respect to these relationships with Bayesian PPs typically higher than parsimony BPs. Our resolution of *S. algira* as a basal lineage sister to the European and Middle Eastern Salamandra contrasts with the placement of *S. algira* as the sister taxon to *S. salamandra* in the mtDNA D-loop tree of Steinfartz et al. (2000), but is concordant with the mtDNA cytochrome *b* results of Barroso and Bogaerts (2003) and Garcia-Paris et al. (2003). However, none of these relationships are particularly well supported, including our new results, indicating that this relationship may be particularly difficult to resolve with mtDNA sequence data.

Our results also provide further insight into the diversification of lineages within the genus *Lyciasalamandra*, a diverse group of salamanders found across the southern coast of Turkey and a small number of Greek islands. Weisrock et al. (2001) sampled Turkish mainland and coastal islands populations across the formerly polytypic species, *Lyciasalamandra luschani*, and demonstrated that it comprised six divergent mitochondrial lineages that likely corresponded to species-level lineages. Veith and Steinfartz (2004) described these six lineages as species along with a seventh species, *Lyciasalamandra helverseni*, from the Greek islands in the Aegean Sea. However, no genetic data has been presented yet for this species. Weisrock et al. (2001) demonstrated that internal branch lengths separating the six Turkish lineages were extremely short and the null hypothesis of a soft molecular polytomy was statistically rejected, suggesting a rapid radiation. Our results indicate that the Greek island species, *Lyciasalamandra helverseni*, represents a seventh divergent lineage with an average ML-corrected sequence divergence with the other six major lineages of 10.65%. Likelihood-ratio tests reveal that internal branches separating the seven divergent *Lyciasalamandra* lineages are not significantly different from zero length (results not shown). These results further suggest that *Lyciasalamandra* diversified rapidly, likley as a result of tectonic collision between the Arabian plate and the southern edge of Anatolia (Weisrock et al., 2001).

4.2. Phylogenetics of Echinotriton, Pleurodeles, and Tylototriton

Within the large newt clade our phylogenetic analyses are congruent with earlier molecular studies (Hayashi and Matsui, 1989; Titus and Larson, 1995; Veith et al., 2004) in placing the southern and southeastern Asian genera *Echinotriton* and *Tylototriton* together with the European and North African genus *Pleurodeles* in a strongly supported clade that forms the

sister lineage to a clade containing the remaining newts (Figs. 1, 2). Nearly all branches within this clade are extremely well supported (Fig. 3). Phylogenetic relationships and patterns of genetic diversity within *Pleurodeles* are similar to the results of Veith et al. (2004) in finding minimal haplotypic divergence between *P. waltl* haplotypes sampled on either side of the Gibraltar Strait.

Our results provide the first assessment of phylogenetic relationships among species of the genera *Echinotriton* and *Tylototriton*. Species of *Echinotriton*, formerly placed in *Tylototriton*, were erected as a new genus in recognition of geographic, morphological, and life history differences (Nussbaum and Brodie, 1982). Our results support the genetic distinction between *Echinotriton* species and *Tylototriton* species (Fig. 3). Relationships among *Tylototriton* species are extremely well supported except for the relationships among *T. kweichowensis*, *T. shanjing*, and *T. verrucosus*. *Tylototriton shanjing* was formerly synonymous with *T. verrucosus*, but was diagnosed as a distinct species based on its unique orange coloration which distinguishes it from the allopatric brown-colored *T. verrucosus* (Nussbaum et al., 1995). Maximum-likelihood corrected sequence divergences between the *T. shanjing* and *T. verrucosus* haplotypes are nearly 6.2%, indicating considerable genetic divergence. The Chinese Hainan island species *T. hainanensis* is placed in a strongly supported clade with an undescribed *Tylototriton* species collected from Vietnam. This undescribed species may represent an allopatric range extension of *T. hainanensis*, but genetic divergences between these samples are comparable to genetic divergences in other *Tylototriton* sister-species comparisons.

4.3. Phylogenetics of Notophthalmus and Taricha

The North American genera *Notophthalmus* and *Taricha* are placed in a clade that forms the sister lineage to all newts excluding *Echinotriton*, *Pleurodeles*, *Salamandrina*, and *Tylototriton* (Figs. 1, 2). This relationship is strongly supported in both the Bayesian and parsimony analyses, although parsimony bootstraps tend to be more conservative in their level of support. Titus and Larson (1995) only weakly recovered *Notophthalmus* and *Taricha* as sister genera and placed this clade as the sister lineage to a clade containing *Cynops*, *Pachytriton*, *Paramesotriton*, and some species of *Triturus*. Our results provide a strongly supported alternative relationship that is more congruent with the allozyme-based phylogeny of Hayashi and Matsui (1989). Relationships among species within *Notophthalmus* and *Taricha* have not previously been explored, although a number of studies have addressed phylogeography within individual species (Gabor and Nice, 2004; Kuchta and Tan, 2005; Reiley, 1990; Tan and Wake, 1995). Within *Notophthalmus*, Bayesian analysis strongly supports the sister relationship of *N. perstriatus* and *N. viridescens* (Fig. 3). Within *Taricha*, *T. granulosa* and *T. torosa* are strongly supported as sister taxa (Fig. 3).

4.4. Phylogenetics of Euproctus, Neurergus, and Triturus

Our results indicate strong support for a large clade containing all species of the genera *Cynops*, *Euproctus*, *Neurergus*, *Pachytriton*, *Paramesotriton*, and *Triturus* (Figs. 1, 2). Within this large clade the genera *Cynops*, *Pachytriton*, and *Paramesotriton* are placed in a strongly supported clade (discussed below). *Neurergus* is also supported as a strongly supported monophyletic group (Steinfartz et al., 2002); however, it is placed as the sister lineage to a lineage of *Triturus vittatus*, which highlights the complexity of relationships among species of the genus *Triturus* and *Euproctus*. Molecular phylogenetic investigation of the evolution of the

genus *Triturus* has received considerable attention (Busack et al., 1988; Giacomo and Baletto, 1988; Halliday and Arano, 1991; McGregor et al., 1990; Zajc and Arntzen, 1999) with some molecular studies indicating that it does not form a monophyletic group (Titus and Larson, 1995; Zajc and Arntzen, 1999). Furthermore, molecular (mtDNA and nuclear rDNA) phylogenetic investigations of the genus *Euproctus* have indicated that it also is not monophyletic and that lineages within these two genera are intertwined with short internal branching events (Caccone et al., 1994; Caccone et al., 1997). A major limitation of these studies has been the use of limited ingroup or outgroup taxon sampling, which has precluded a complete view of the evolution of *Euproctus* and *Triturus*.

Through nearly complete taxon sampling our results robustly resolve nonmonophyletic histories for both *Euproctus* and *Triturus* (Figs. 1, 2, 4). *Triturus* species are resolved into four main lineages: (1) A clade containing all species of the *T. cristatus* species group (*T. carnifex*, *T. cristatus*, *T. dobrogicus*, *T. karelini*, and *T. pygmaeus*); (2) a clade containing the *T. vulgaris* species group (*T. montandoni*, and *T. vulgaris*) and *T. boscai*; (3) a *T. alpestris* clade, and (4) a clade containing *T. vittatus* and all species of the genus *Neurergus*. As in previous studies (Caccone et al., 1994, 1997), the Mediterranean island *Euproctus* species, *E. montanus* (Corsica) and *E. platycephalus* (Sardinia) form a strongly supported clade. This lineage basally diverges from a large and diverse newt clade containing *E. asper* and *Cynops*, *Euproctus*, *Pachytriton*, *Paramesotriton*, and *Triturus*. *Euproctus asper* is placed as the sister lineage to a clade containing all species of the *T. cristatus* species group. Relationships among the above described lineages of *Euproctus* and *Triturus* and the *Cynops-Pachytriton-Paramesotriton* clade are robustly supported in the Bayesian analysis with many branches receiving PPs of 0.99-1.0 (Fig. 1). Parsimony analysis finds a congruent topology, but with lower levels of branch support (Fig.

2). Nonetheless, monophyly of the *Euproctus* and *Triturus* are both strongly rejected under the conservatively-biased SH test, and nonmonophyly of *Euproctus* is nearly rejected under the Templeton test (Table 2).

4.5. Phylogenetics of Cynops, Pachytriton, and Paramesotriton

Our results are in strong agreement with previous molecular studies in resolving the genera *Cynops*, *Pachytriton*, and *Paramesotriton* as a monophyletic group (Chan et al., 2001; Hayashi and Matsui, 1989; Titus and Larson, 1995). Relationships within this clade have been more difficult to resolve. *Pachytriton* is the only genus that receives robust support for monophyly in our analyses (Fig. 4), consistent with the findings of Chan et al. (2001) that *Pachytriton* species are highly distinct in morphology from species of *Cynops* and *Paramesotriton*. Using mtDNA sequences from two of the six extant species Chan et al. (2001) resolved *Cynops* to be a paraphyletic genus, with *C. pyrrhogaster* forming the sister lineage to a clade of *Pachytriton* and *Paramesotriton*. Our results, which include sequence data from five of seven *Cynops* species, resolve the genus as a monophyletic group in both Bayesian and parsimony analyses; however, this relationship is poorly supported by both PPs and BPs (Fig. 4).

The genus *Paramesotriton* contains divergent genetic lineages that are not resolved as a monophyletic group (Fig. 4). Nonmonophyly of the genus results from the placement of *Paramesotriton laoensis*, a recently described species from Laos (Stuart and Papenfuss, 2002), as the sister lineage to a well supported clade containing the genus *Pachytriton* and all remaining species of *Paramesotriton*. *Paramesotriton laoensis* is morphologically distinctive from other *Paramesotriton* species in a number of characters, especially in skin coloring, wart and gland skin coverage, and in having an undifferentiated tongue pad (similar to that of *Pachytriton*)

(Stuart and Papenfuss, 2002). It is morphologically similar to other species of *Paramesotriton* in its skull morphology and vertebral number (12), which are the primary characters used to place *P. laoensis* in the genus *Paramesotriton*. Our results suggest that these shared characters likely represent symplesiomorphies and that *P. laoensis* is not a member of the genus *Paramesotriton*. Alternatively, it is resolved as a distinct evolutionary distinct lineage with ML-corrected sequence divergences between other species of *Paramesotriton* (avg=18.1%) that are similar to sequence divergence comparisons with species of the genera *Pachytriton* (avg=17.7%) and *Cynops* (20.4%).

The remaining species and samples of *Paramesotriton* are strongly supported as a monophyletic group with a Bayesian PP of 1.0 (Fig. 4) and relationships are similar to those reconstructed by Lu et al. (2004). Our data set contains a number of recently collected samples that could not be morphologically diagnosed as recognized species, but which are phylogenetically resolved as minimally divergent lineages from other recognized species. Samples from separate and allopatrically disjunct (TED, IS THIS TRUE?) localities of the Chinese newt, Paramesotriton *chinensis*, are characterized by divergent non-monophyletic mtDNA haplotypes, indicating that this species may be comprised of divergent evolutionary lineages.

4.6 Tempo of salamandrid diversification

Our results do not support the hypothesis that the Salamandridae went through periods of rapid lineage formation (i.e. radiations). Our LTT plots and γ statistic measures exhibit patterns consistent with a slightly higher rate of lineage formation early in salamandrid history; however, the CR test is unable to reject the null hypothesis of constant rates of lineage formation across

the recoverable history of the Salamandridae. Furthermore, the relative cladogenesis statistic does not reveal any internal branches in the Bayesian consensus tree that have given rise to a disproportionate amount of subsequent lineages. It seems unlikely that our results are artifactual, given that we include nearly all recognized species. Failure to include cryptic or undiscovered lineage diversity (e.g. *Paramesotriton laoensis*) could negatively bias our measurements of γ, leading to incorrect inferences of a historically more rapid rate of lineage formation, or in a more recent slowdown (Pybus et al., 2002). Future inclusion of additional cryptic lineages is expected to further straighten salamandrid LTT curves, and strengthen our conclusions of constant rates of lineage diversification.

Our results indicate that the evolution of a substantial amount of behavioral, ecological, and morphological character variation in the Salamandridae has not coincided with increased rates of speciation and lineage formation. Much attention has been placed on disparity in trophic morphology in salamandrids, which has been characterized as an important adaptive factor in the evolution of major salamandrid groups (the terrestrial genera *Chioglossa*, *Lyciasalamandra*, *Mertensiella*, *Salamandra*, and *Salamandrina* vs. the remaining aquatic or amphibious genera) (Özeti and Wake, 1969; Titus and Larson). The evolution of a hyobranchial feeding morphology for aquatic and amphibious salamandrids is considered to be the derived condition within the family (Titus and Larson, 1995) and interestingly, this correlates with the most species-rich clade in salamandrid phylogeny (Fig. 1). Yet, our phylogenetic hypotheses do not produce a pattern that would indicate an increased rate of lineage formation within this clade. Changes in trophic morphology associated with feeding in terrestrial versus aquatic environments may indeed have been be important adaptations for salamandrid species; however, they do not appear to have been influential in driving the formation of new species within these clades.

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Figure Legends

Figure 1. Bayesian majority-rule consensus phylogram of trees sampled from the posterior distribution of a tri-partitioned analysis of the *tRNA^{Leu}* -COI mtDNA sequence data. Numbers above or below branches are posterior probabilities. Phylogenetic relationships in the unpartitioned analysis did not differ substantially from those of the partitioned analysis. Relationships within major clades are collapsed for easier presentation and are presented in detail in Figures 3 and 4. The thick black branch leads to *Salamandrina terdigitata*.

Figure 2. Parsimony phylogram resulting from analysis of the *tRNA^{Leu}* -COI mtDNA sequence data. Numbers above or below branches represent bootstrap values. Relationships within major clades are collapsed for easier presentation and are presented in detail in Figures 3 and 4. The thick black branch leads to *Salamandrina terdigitata*.

Figure 3. Phylogenetic relationships for major clades identified in figures 1 and 2. This includes relationships for (A) *Lyciasalamandra* and *Salamandra*, (B) *Echinotriton*, *Tylototriton*, and *Pleurodeles*, and (C) *Notophthalmus* and *Taricha*. Branch lengths and topology are from the Bayesian majority-rule consensus phylogram. Numbers above branches are Bayesian posterior probabilities. Numbers below branches are parsimony bootstrap values.

Figure 4. Phylogenetic relationships for major clades identified in figures 1 and 2. This includes relationships for (D) *Triturus boscai* and the *Triturus vulgaris* species group, (E) *Neurergus* and *Triturus vittatus*, (F) the *Triturus cristatus* species group, and (G) *Cynops, Pachytriton,* and *Paramesotriton*. Branch lengths and topology are from the Bayesian majority-rule consensus

phylogram. Numbers above branches are Bayesian posterior probabilities. Numbers below branches are parsimony bootstrap values.

Figure 5. Lineage-through-time plots for 10 trees sampled from the Bayesian posterior distribution. The y-axis (number of reconstructed lineages) is presented in logarithmic format.

Table 1. Taxon sampling used in this study

Taxon	Museum/GenBank Number	Locality
Necturus alabamensis	MVZ187705	Walton Co., FL, United States
Ambystoma trigrinum	MVZ187202	Oakland Co., MI, United States
Eurycea wilderae	KHK188.8	
Phaegnathus hubrichti	MVZ173507/FC13612	Butler Co., AL, United States
Dicamptodon tenebrosus	MVZ187929	Trinity Co., CA, United States
Chioglossa lusitanica	MVZ230958/AF29662	San Martin de Luina, Asturias, Spain
Cynops cyanurus	MVZ219759/S11637	Chuxiong, Yunnan Prov., China
Cynops ensicauda	TP24749	
Cynops orientalis	JF259	Fujian Province
Cynops orientalis	TP25011	
Cynops orphicus	TP26273	
Echinotriton andersoni	DW82	
Echinotriton chinhaiensis	TP26195	
Euproctus asper	EAES3	From Mario
Euproctus montanus	1978.584	
Euproctus platycephalus	DWW1225	
Mertensiella caucasica	MVZ218721/AF296621	~10 km SSE of Borzhomi, Georgia.
Neurergus crocatus	TP27066	
Neurergus kaiseri	TP26965	
Neurergus microspilotus	TP26094	
Neurergus strauchii	TP27045	
Neurergus strauchii barani	TP27051	
Notophthalmus meridionalis	DW80	
Notophthalmus perstriatus	DW71	Ocala National Forest, Putnam Co., FL, United States
Notophthalmus viridescens	MVZ230959/AF29661	St. Charles Co., Missouri, United States
Pachytriton brevipes	DW75	

Pachytriton labiatus	CAS194298/AF296618	Jiaxing Prefecture, Zhejiang Province, China
Pachytriton sp.	JF269	
Paramesotriton caudopunctatus	TP28001	
Paramesotriton chinensis	TP24995	
Paramesotriton chinensis	TP25035	
Paramesotriton deloustali	TP23630	
Paramesotriton fuzhongensis	TP25043	
Paramesotriton gaunxiensis	MVZ220905/S12716	Linming Co.; Guangxi Zhuang Autonomous Region, China
Paramesotriton hongkongensis	TP25836	
Paramesotriton hongkongensis	TP24839	
Paramesotriton hongkongensis	TP24846	
Paramesotriton laoensis	FMNH255452	
Paramesotriton sp.	ROM35433	
Paramesotriton sp.	FMNH259125	
Paramesotriton sp.	TP28303	
Pleurodeles poireti	TP27330	
Pleurodeles waltl	MVZ162384/FC11135	Rabat, Morocco
Pleurodeles waltl	SDB1750	Spain
Salamandra algira		
Salamandra atra	TP27291	
Salamandra atra aurorae	TP27292	
Salamandra corsicae		
Salamandra i. infraimmaculata	MVZ230199/AF296624	Harbiye, Hatay Prov., Turkey
Salamandra infraimmaculata semenovi	TP26145	
Salamandra lanzai	TP27293	
Lyciasalamandra antalyana	MVZ230190/AF296625	Hurma Köyü, Antalya Prov., Turkey
Lyciasalamandra atifi	MVZ230197/AF296629	Fersin Köyü, Antalya Prov., Turkey
Lyciasalamandra billae	MVZ230184/AF296626	Bnynk Calticak Beach, Antalya Prov., Turkey
Lyciasalamandra fazilae	MVZ230159/AF296627	Domuz Adasi, Fethiye Bay, Mugla Prov., Turkey

Lyciasalamandra flavimembris	MVZ230148/AF296635	Cicekli Köyü, Mugla Prov., Turkey
Lyciasalamandra helverseni	TP26395	Karpathos Island
Lyciasalamandra luschani luschani	MVZ230165/AF296632	Dodurga Köyü, Mugla Prov., Turkey
Lyciasalamandra luschani basoglui	MVZ230171/AF296633	Nandarlar Köyü, Antalya Prov., Turkey
Lyciasalamandra luschani finikensis	MVZ230177/AF296631	Finike, Antalya Prov., Turkey
Salamandra salamandra	MVZ186046/AF296622	Cadiz, Andalusia, Spain.
Salamandrina terdigitata	MVZ178849/S7539	Cardoso, Stazzemese, Prov. Lucca Toscana Region, Italy
Taricha granulosa	ED	
Taricha granulosa	MVZ173374/S6490	Tehama Co., California, USA
Taricha rivularis	MVZ158853/S6517	Mendocino Co., California, USA
Taricha torosa	TP25072	
Taricha torosa	TP25697	
Triturus alpestris alpestris	DWW1168	Sukhodol, Opolian Highland, Lvov Province, Ukraine
Triturus alpestris cyreni	DWW337 (L12)	Lloroza, Cantabria, Spain
Triturus boscai	DWW336	Tabuyo, Leon, Spain
Triturus carnifex carnifex	DWW1186	Venice, North-East Italy
Triturus carnifex macedonicus	DWW1189	Donja Locanj, Montenegro, Yugoslavia
Triturus cristatus	DWW1199	Chur, Udmurtia, Volga River Basin, Russia
Triturus dobrogicus macrosomus	DWW1196	Minai. Transcarpathians Province, Ukraine
Triturus helveticus	DWW1155	
Triturus karelini	RM7627	Azerbaijan
Triturus karelini	RM7094	Georgia
Triturus montandoni	TP26567	
Triturus montandoni	DWW1158	Sukhodol, Opolian Highland, Lvov Province, Ukraine
Triturus marmoratus	MVZ191887	Barcelona Prov., Catalonia, Spain
Triturus marmoratus	DWW334	Arrillor, Alava, Spain
Triturus pygmaeus	DWW335	Pelahustan, Toledo, Spain
Triturus vittatus	RM7611	
Triturus vittatus ophriticus	DWW1101	Psebai, Krasnador Territory, Russian North-West Caucasus

Triturus vulgaris	RM7631	
Triturus vulgaris	TP26609	
Triturus vulgaris lantzi	DWW1117	Stavropol, Russian North-West Caucasus
Tylototriton asperrimus	TP26278	
Tylototriton hainanensis	TP24824	
Tylototriton kweichowensis	TP25555	
Tylototriton shanjing	MVZ219763/S11641	Jingdong, Yunnan Province, China
Tylototriton taliangensis	CAS195126/AF296617	Liangshan Yizu Autonomous Pref., Sichuan Province, China
Tylototriton verrucosus	NO2804	
Tylototriton wenxianensis	TP26244	
Tylototriton sp.	ROM35330	

Table 2. Topology test results

Alternative Hypothesis	SH Test Delta lnL (p-value)	Templeton Test Delta (p-value)
Salamandrina sister lineage to remaining Newt clade	2.006 (p=0.36)	—
Salamandrina sister lineage to "true" salamander clade	—	
Triturus Monophyly	53.973 (p=0.003)	25 (p≤0.1338)
Euproctus Monophyly	63.537 (p<0.001)	27 (p≤0.0686)

Table 3. Mean model parameter estimates for each partition of the $tRNA^{Leu}$-COI genic region calculated from the Bayesian posterior distribution.

Model Parameter	Total Partition	ND1	ND2+COI	tRNAs
Ti:Tv	—	—	—	
G↔T	1	1	1	—
C↔T	5.737	7.788	3.916	—
C↔G	0.935	1.335	0.828	—
A↔T	0.533	0.7	0.365	—
A↔G	13.292	17.157	9.986	—
A↔C	0.807	1.078	0.546	—
Freq. A	0.387	0.373	0.4	0.392
Freq. C	0.248	0.254	0.247	0.212
Freq. G	0.067	0.069	0.058	0.151
Freq. T	0.297	0.303	0.295	0.245
Prop. Invar.	0.275	0.316	0.24	0.18
α	0.693	0.733	0.802	0.372

Table ?. Gamma Statistics calculated for trees from the Bayesian posterior distribution.

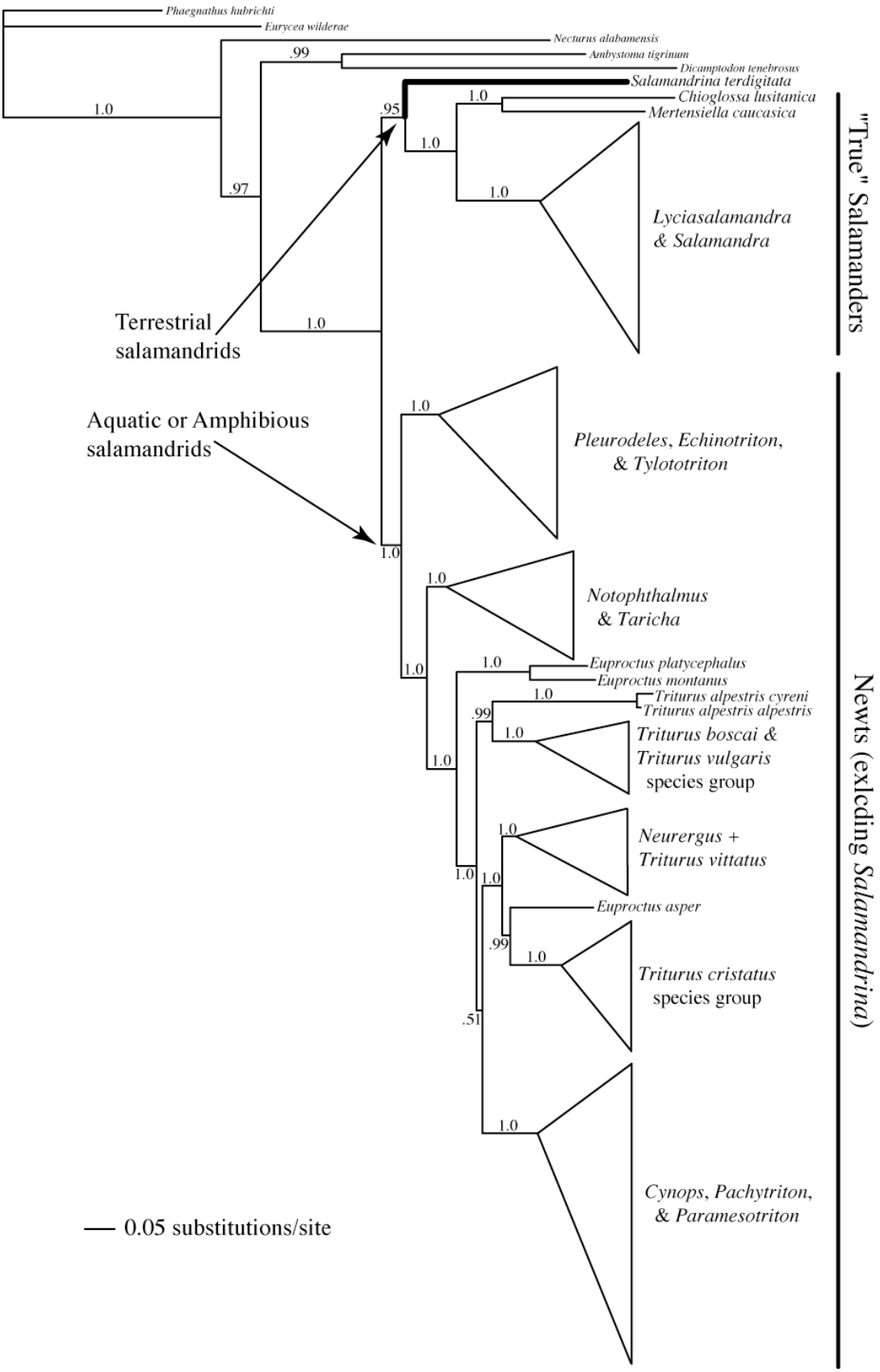
Posterior Tree	γ (Full Tree)	γ (2/3 Tree)
Tree 1	-0.3179	-0.9831
Tree 101	-0.5139	-0.6239
Tree 201	0.2209	-0.5452
Tree 302	-0.7317	-0.8437
Tree 401	-0.1910	-0.8419
Tree 501	-0.1776	-1.0221
Tree 601	0.4539	-1.0496
Tree 700	-0.2074	-0.6293
Tree 801	-0.2913	-1.1869
Tree 900	0.3586	-1.2302
Average	-0.1397	-0.8956

Appendix 1

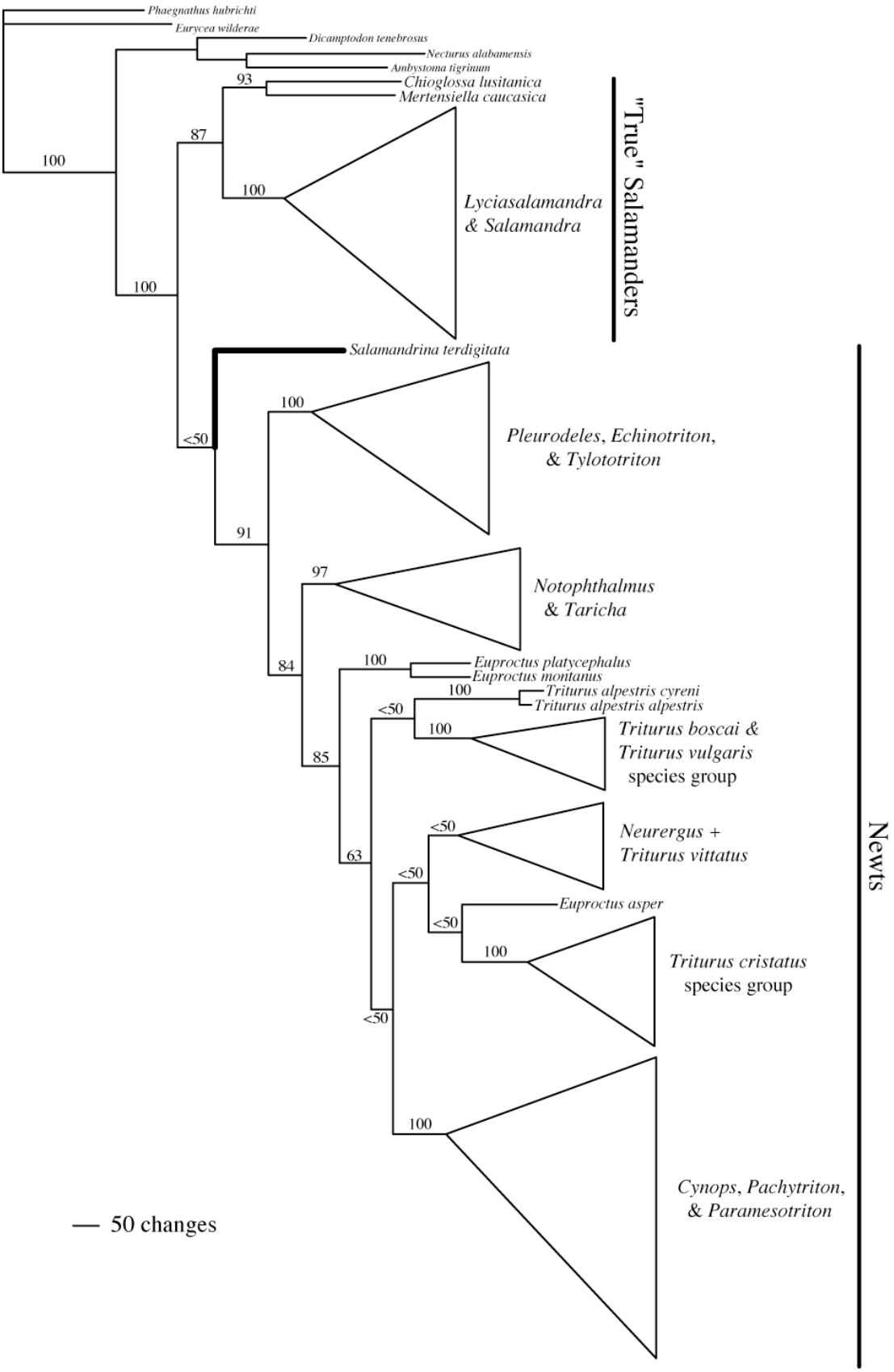
Previously published mtDNA sequences used in this study are listed below. When available, sequences are marked with their GenBank accession number. Not all 12S-tRNA^{Val}-16S sequences are accessioned in GenBank. Sequences published by Titus and Larson (1996) and Zajc and Arntzen (1999) are marked with TL96 and ZA99, respectively. 12S-tRNA^{Val}-16S sequences: *Phaeognathus hubrichti*, TL96; *Eurycea wilderae*, TL96; *Necturus maculosus*, TL96; *Ambystoma tigrinum*, TL96, *Dicamptodon tenebrosus*, TL96; *Chioglossa lusitanica*, TL96; *Cynops ensicauda*, TL96; *Cynops pyrrhogaster*, TL96; *Euproctus asper*, TL96; *Euproctus montanus*, U04696; *Euproctus platycephalus*, U04698; *Mertensiella caucasica*, TL96; *Neurergus crocatus*, AY147246, *Neurergus kaiseri*, AY147250; *Neurergus microspilotus*, AY147248; *Neurergus strauchii strauchii*, TL96; *Neurergus strauchii barani*, AY147244; *Notophthalmus viridescens*, TL96; *Pachytriton labiatum*, TL96; *Paramesotriton deloustali*, TL96; *Pleurodeles waltl*, TL96; *Salamandra atra*, TL96; *Salamandra salamandra*, TL96; *Salamandra luschani*, TL96; *Salamandrina terdigitata*, TL96; *Taricha granulosa*, TL96; *Triturus alpestris*, TL96; *Triturus boscai*, ZA99; *Triturus carnifex*, U04702; *Triturus cristatus*, ZA99; *Triturus karelini*, TL96; *Triturus marmoratus*, AY147252; *Triturus montandoni*, ZA99; *Triturus vittatus*, ZA99; *Triturus vulgaris*, U04704; *Tylototriton taliangensis*, TL96; *Tylototriton verrucosus*, TL96. Cytochrome *b* sequences: *Ambystoma tigrinum*, Z11640; *Eurycea wilderae*, AF252379; *Chioglossa lusitanica*, AF329300; *Cynops cyanurus*, AF295682; Cynops pyrrhogaster, AF295681; *Euproctus asper*, U55945; *Euproctus montanus*, U55946; *Euproctus platycephalus*, U55947; *Mertensiella caucasica*, AF170013; *Neurergus crocatus*, AY336661; *Notophthalmus perstriatus*, AF380362; *Notophthalmus viridescens*, L22882; *Pachytriton labiatum*, AF295679; *Paramesotriton caudopunctatus*, AF295675; *Paramesotriton deloustali*,

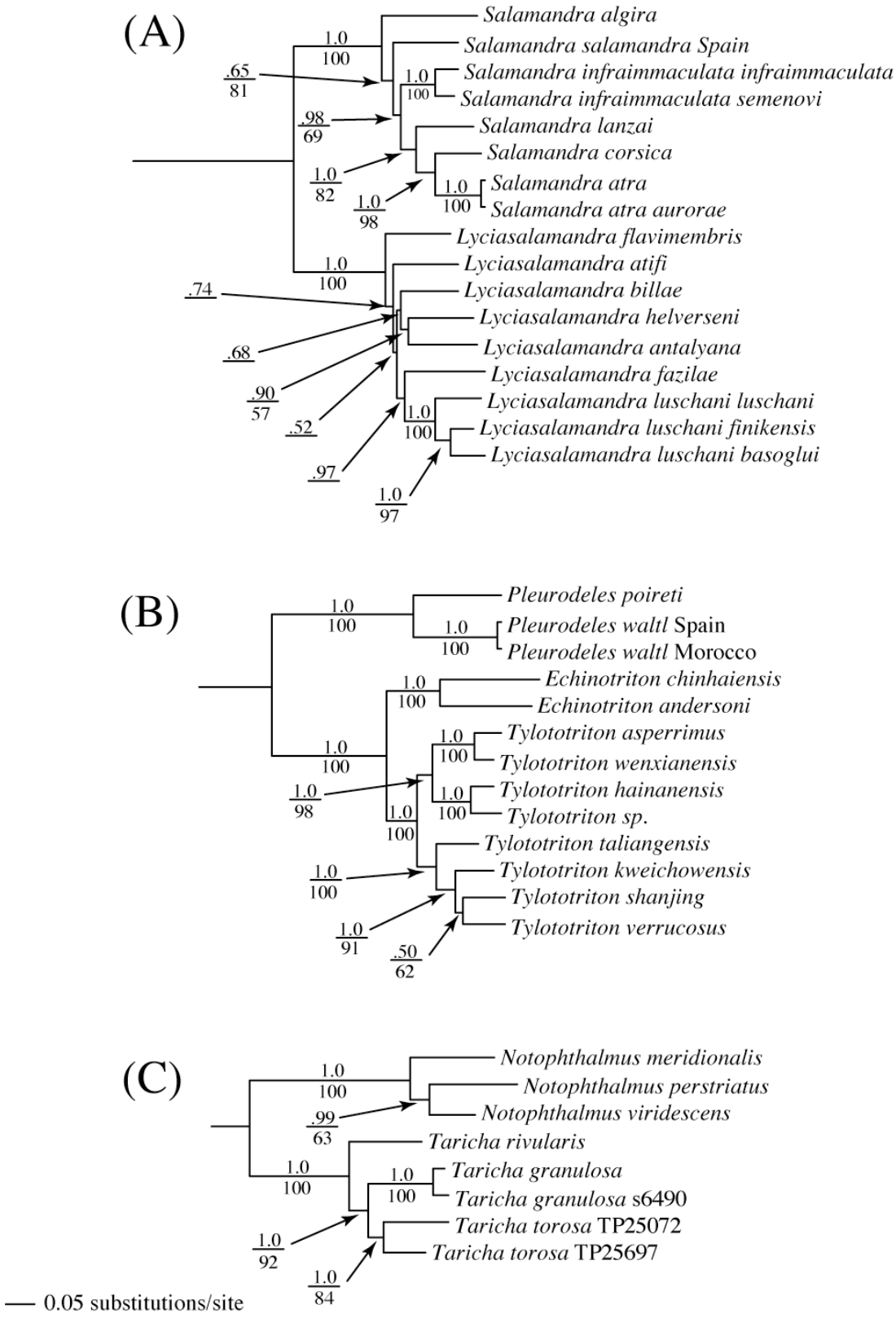
AF295671; *Paramesotriton guanxiensis*, AF295673; *Paramesotriton hongkongensis*, AF295677; *Pleurodeles poireti*, AY336644; *Pleurodeles waltl*, U55950; *Salamandra salamandra*, AY336658; *Salamandra algira*, AY247734; *Salamandra atra atra*, AY042786; *Salamandra atra aurorae*, AY042784; *Salamandra lanzai*, AY196284; *Salamandra luschani*, AF154053; *Taricha granulosa*, AF295683; *Taricha rivularis*, L22713; *Taricha torosa*, L22708; *Triturus carnifex*, U55949; *Triturus marmoratus*, AY046081; *Triturus pygmaeus*, AY046082; *Triturus vittatus*, AY336659; *Triturus vulgaris*, U55948; *Tylototriton taliangensis*, AF295684; *Tylototriton verrucosus*, AF295685.

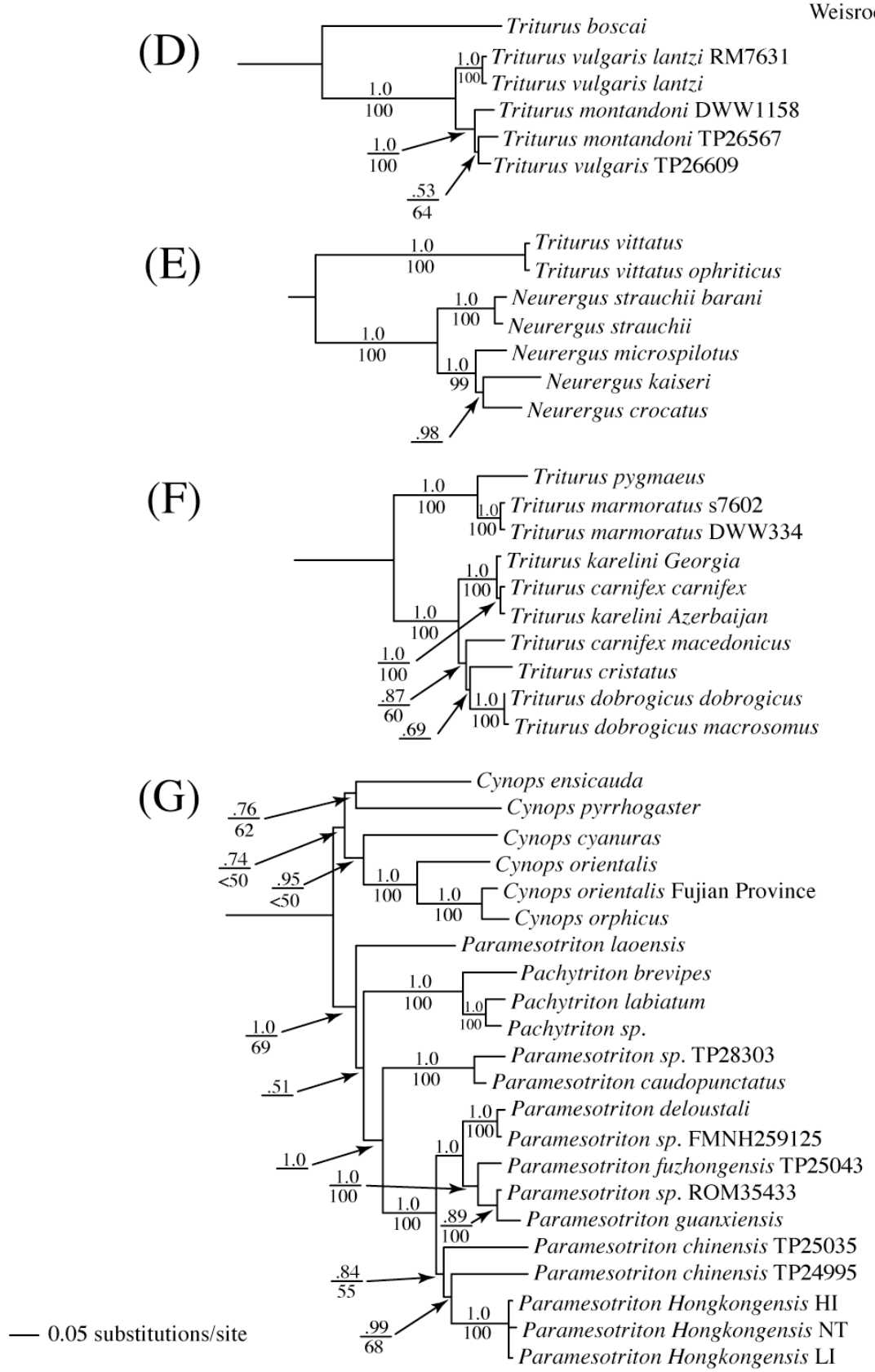
Weisrock et al. Figure 1



Weisrock et al. Figure 2




(A)
Salamandra algira
Salamandra salamandra Spain
Salamandra infraimmaculata infraimmaculata
Salamandra infraimmaculata semenovi
Salamandra lanzai
Salamandra corsica
Salamandra atra
Salamandra atra aurorae
Lyciasalamandra flavimembris
Lyciasalamandra atifi
Lyciasalamandra billae
Lyciasalamandra helverseni
Lyciasalamandra antalyana
Lyciasalamandra fazilae
Lyciasalamandra luschani luschani
Lyciasalamandra luschani finikensis
Lyciasalamandra luschani basoglui
(B)
Pleurodeles poireti
Pleurodeles waltl Spain
Pleurodeles waltl Morocco
Echinotriton chinhaiensis
Echinotriton andersoni
Tylototriton asperrimus
Tylototriton wenxianensis
Tylototriton hainanensis
Tylototriton sp.
Tylototriton taliangensis
Tylototriton kweichowensis
Tylototriton shanjing
Tylototriton verrucosus
(C)
Notophthalmus meridionalis
Notophthalmus perstriatus
Notophthalmus viridescens
Taricha rivularis
Taricha granulosa
Taricha granulosa s6490
Taricha torosa TP25072
Taricha torosa TP25697
0.05 substitutions/site


(D)
Triturus boscai
Triturus vulgaris lantzi RM7631
Triturus vulgaris lantzi
Triturus montandoni DWW1158
Triturus montandoni TP26567
Triturus vulgaris TP26609
(E)
Triturus vittatus
Triturus vittatus ophriticus
Neurergus strauchii barani
Neurergus strauchii
Neurergus microspilotus
Neurergus kaiseri
Neurergus crocatus
(F)
Triturus pygmaeus
Triturus marmoratus s7602
Triturus marmoratus DWW334
Triturus karelini Georgia
Triturus carnifex carnifex
Triturus karelini Azerbaijan
Triturus carnifex macedonicus
Triturus cristatus
Triturus dobrogicus dobrogicus
Triturus dobrogicus macrosomus
(G)
Cynops ensicauda
Cynops pyrrhogaster
Cynops cyanuras
Cynops orientalis
Cynops orientalis Fujian Province
Cynops orphicus
Paramesotriton laoensis
Pachytriton brevipes
Pachytriton labiatum
Pachytriton sp.
Paramesotriton sp. TP28303
Paramesotriton caudopunctatus
Paramesotriton deloustali
Paramesotriton sp. FMNH259125
Paramesotriton fuzhongensis TP25043
Paramesotriton sp. ROM35433
Paramesotriton guanxiensis
Paramesotriton chinensis TP25035
Paramesotriton chinensis TP24995
Paramesotriton Hongkongensis HI
Paramesotriton Hongkongensis NT
Paramesotriton Hongkongensis LI
0.05 substitutions/site

Weisrock et al. Figure 5